DOCUMENT-IDENTIFIER: US 5681571 A US-PAT-NO: 5681571 TITLE: Immunological tolerance-inducing agent STATE ZIP CODE COUNTRY DATE-ISSUED: October 28, 1997 INVENTOR-INFORMATION: UZERKINSKY; UECII GOTEDORG NIA NIA SEX 424/810 ,514/885 US-CL-CURRENT: 424/236.1,424/241.1 ,424/275.1 ,424/282.1 ,424/810 ,514/885 NAME Holmgren; Jan An immunological tolerance-inducing agent comprising a mucosa-binding Czerkinsky, Cecil molecule linked to a specific tolerose in an individual accident a inducing immunological tolerance in an individual against a specific antigen, including immunological colerance in an incividual against a specific antigen, which causes an unwanted immune response in said individual including hapten, which causes an unwanted immune response in said individual including hapten, which causes an unwanted immune response in said individual including hapten, which causes an unwanted immune response in said individual including hapten, which causes an unwanted immune response in said individual including hapten. ,530|868 ABSTRACT: mount of an immunological tolerance indicate from immunologically effective winipinany aunimianauvii by a mucusan route of an immunological tolerance-inducing agent of the invention to said amount of an immunological tolerance-inducing agent of the invention to said individual, is described. 27 Claims, 0 Drawing figures

The way in which the specific tolerogen is linked to a mucosa-binding molecule Exemplary Claim Number: 1 in an immunological tolerance and add malacile and add ma an an minimunous contraince mounting again or the invention to more their respective as long as said tolerogen and said molecule can perform their respective as long as said tolerogen and said molecule can be discounted by a said tolerogen. The short mark he indicated to said after discounted to as jury as saw were way be linked to each other directly by simple chemical function. Thus, they may be linked to each other directly by simple chemical procedures. Chemical procedures to couple proteins such as the B subunit of procedures. Unermod procedures to couple procedures and as the state of the thermolabile enterotoxin of Escherichia coli (LTB) cholera toxin (CTB) or the thermolabile enterotoxin of the characteristic and as the characteristic to lipids, haptens, carbohydrates, nucleic acids as well as to other proteins including antibodies and synthetic peptides are well known in the art (e.g. see Including annuous and symmetry populars are well known in the art [e.g. 500]. Carlsson, J. et al. 1978. Biochem. J. 173:723-737; Cumber, J. A. et al. 1985. Valissoli, J. et al 1810. Divulelli. J. 113.143-131, Juliliosi, J. N. et al. 180. Methods in Enzymology 112:207-224; Walden, P. et al. 1986. J. Mol. Cell INTERIOUS III EIIZYIIIUIUUYY 112.2017224, vvalueli, F. et al. 1300. J. Nill. Acad. Sci. Immunol. 2:191-197; Gordon, R. D. et al. 1987. 7 4000 (USA) 84:308-312; Avrameas, S. and Temynok, T. 1969. Immunochemistry 6:53; Joseph, K. C., Kim, S. U., Stieber, A., Gonatas, N. K. 1978. Proc. Natl. лизери, п. U., пин, э. U., эненен, п., энинака, и. п. наи. наи. Асаd. Sci. USA 75:2815-2819; Middlebrook, J. L. and Kohn, L. D. (eds): 1981. now. Jul. UJA 13.2013-2013, Milluleuruun, J. L. aliu nuriii, L. D. (803). 1301.

Receptor-mediated binding and internalization of toxins and hormones.

Proce Now York and 244 250. The telegraph and of the fined condition to Press, New York, pp 311-350). The tolerogen can also be fused genetically to the CTB (or LTB) 2000 (conchot I changed and I allowed I also the CTB (or LTB) 2000 (conchot I changed III) the CTB (or LTB) gene (Sanchez, J., Svennerholm, A-M and Holmgren, J. 1988. Genetic fusion of a non-toxic heat-stable enterotoxin-related deca-peptide Generic rusion of a non-road free relationship to cholera toxin B subunit. FEBS Letters 241: 110-114) and the antigen to cholera toxin B subunit. anuyen to chose a town to suppressed in a suitable expression system, such resulting chimeric gene then be expressed in a suitable expression system. resulting criminers gene treat the expressed in a surface inducing agent as a bacteria, a yeast of a virus. Alternatively, the tolerance inducing agent as a pacteria, a yeast or a virus. Alternatively, the lorerative mountains agent as a pacteria, a yeast or a virus. Alternatively, the loreration which is then chemically counted to the advanced of a nucleic acid sequence (DNA or RNA) or a synthetic may comprise a fragment of a nucleic acid sequence (DNA or RNA) or a synthetic may comprise a fragment of a nucleic acid sequence (DNA or RNA) or a synthetic polynucleotide encoding the tolerogen which is then chemically coupled to the pulyinucleusine encountry the consoits of colle from host miscord from to control the consoits of colle from host miscord from to control the consoits of colle from host miscord from to control the consoits of colle from host miscord from the consoits of collections of collections of the consoits of collections of the collections then taken of the capacity of cells from host mucosal tissues to ensure transcription and/or translation of the corresponding gene into a mature uanovipuon anuor uanolauon on une ooneoponumy yene וווט a maiure Gene-transfer for therapy protein (Rohrbaugh, M. L. and McGowan, J. J. 1993. Gene-transfer

1-1 infection. Ann. N.Y. Acad. Sci. Vol 685, pp and Felgner, P. L., 1993. Direct gene-transfer for munization. Trends in Biotechnology Vol 11 No. 5, pp L. Hunt, L. A., Webster, R. G. 1993. Protection against challenge by immunization with a ing plasmid DNA. <u>Vaccine</u> 11:957-960; Martinon, F.,

and Meulien, P. 1993. Eur. J. Immunol. 23:1719-1722). Yet other alternative presentation forms could consist in the incorporation of the tolerogen or its nucleic acid precursor into a protective vehicle such as a liposome or equivalent biodegradable vesicles onto which the mucosa-binding substance had been or shall be attached allowing efficient binding of the tolerogen-containing vehicle to a mucosal surface for improved tolerogenic efficacy. With this type of presentation form, the tolerogen may be either free or linked to another molecule.

BSPR:

Recombinant cholera toxin B subunit (CTB) was produced in a mutant strain of Vibrio cholerae deleted of the cholera toxin genes and transfected with a plasmid encoding the CTB subunit (Sanchez, J. and Holmgren, J. 1989. Recombinant system for over-expression of cholera toxin B subunit in Vibrio cholerae as a basis for vaccine development. Proc. Natl. Acad. Sci. USA 86:481-485). Recombinant B subunit of Escherichia coli heat-labile enterotoxin (LTB) was produced similarly in a mutant strain of Vibrio cholerae deleted of the cholera toxin genes and transfected with a plasmid encoding E. coli LTB (Hirst, T. R., Sanchez, J. Kaper, J. B., Hardy, S. J. S, and Holmgren, J. 1984. Mechanism of toxin secretion by Vibrio cholerae investigated in strains harbouring plasmids that encode heat-labile enterotoxins of Escherichia coli. Proc. Natl. Acad. Sci. USA 81:7752-7756). In these expression Systems, CTB and LTB are recovered from bacterial growth media as secreted proteins. Bacterial cultures were centrifuged at 8000 rev per min for 20 min and the supernatants were collected and adjusted to pH 4.5 with dilute HCl. After precipitation with hexametaphosphate (final concentration 2.5 g/l) for 2 hours at 23.degree. C. followed by centrifugation at 8000 rev per min, the pellets were dissolved with 0.1M sodium phosphate buffer, pH 8.0 and dialysed against 0.01M phosphate-buffered saline, pH 7.2, The dialysate was then centrifuged at 15 000 rev per min to remove residual insoluble material and the supernatant was further clarified by filtration through a 0.22 .mu.m filter (Millipore, Bedford, Mass.). Finally, CTB and LTB were purified by standard gel filtration chromatography through columns of Sephadex G-100 (Pharmacia, Sweden).

CLPR:

1. A method of inducing immunological tolerance in a mammal to a T-cell-associated immunological response, which comprises administering by a mucosal route to a mammal suffering from or prone to a T-cell associated disease an immunological tolerance-inducing agent, wherein said agent comprises (i) a mucosa-binding molecule selected from the group consisting of the B subunit of cholera toxin and the B subunit of heat-labile enterotoxin of Escherichia coli, linked to (ii) a specific tolerogen associated with said T-cell associated immune response, and wherein said agent is administered in an amount and for a time effective to induce tolerance against said T-cell associated immune response.

CLPR:

CT & LTII

(FILE 'HOME' ENTERED AT 16:56:59 ON 26 FEB 2002)

L1 L2	Ę	LINE, BIOSIS, CAPLUS, EMBASE' ENTERED AT 16:57:18 ON 26 FEB 2002 S ENTEROTOXINS AND DNA VACCINE B DUP REM L1 (2 DUPLICATES REMOVED)
L3	885	S ENTEROTOXINS AND (IMMUNIZATION OR VACCINE)
L4	C	S L3 AND (CHLOERA TOXIN B/A2)
L5	C	S L3 AND (CHLOERA TOXIN)
L6		S CHLOERA TOXIN B
L7	226	S S CHOLERA TOXIN AND L3
$\Gamma8$	45	S L7 AND SUBUNITS
L9	27	DUP REM L8 (18 DUPLICATES REMOVED)
L10	12	S L9 AND 1995-2000/PY
L11	6	S HEAT-LABILE TOXIN II
L12	1	S L3 AND L11
L13	3772	S RUSSELL M?/AU OR CONNELL T?/AU
L14	13	S L3 AND L13
L15	4	DUP REM L14 (9 DUPLICATES REMOVED)

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7	3	"5800821"	USPAT;	Time stamp 2002/02/26 18:07
			US-PGPUB:	2002/02/26 18:07
			EPO; JPO;	
			DERWENT	
1	2	"9958145"	USPAT:	2002/02/26 18:17
			US-PGPUB;	2002/02/20 10:17
			EPO; JPO;	
			DERWENT	
13	237	enterotoxin same vaccine	USPAT:	2002/02/26 18:18
			US-PGPUB:	2002/02/20 10.10
			EPO; JPO;	
			DERWENT	
19	29	heat\$labile with II	USPAT;	2002/02/26 18:19
			US-PGPUB:	2002/02/20 18:19
			EPO; JPO;	
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31	7	((enterotoxin same vaccine) and (heat\$labile with II)) and	USPAT;	2002/02/20 40:40
		subunit\$	US-PGPUB;	2002/02/26 18:19
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37	318	cholera adj toxin adj B	DERWENT	2000/00/00 40 04
İ	ĺ		USPAT;	2002/02/26 18:21
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		Community of the same AZ/D	USPAT;	2002/02/26 18:21
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49	245	(cholera adj toxin adj B) same subunit\$		2002/20/20 40 00
		t and the same and an arrangement	USPAT;	2002/02/26 18:22
			US-PGPUB;	
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67	2	(cholera adj toxin adj B) same subunit\$ same adjuvant same	DERWENT USPAT:	2002/02/02 40 02
		expression	US-PGPUB;	2002/02/26 18:22
		·	EPO; JPO;	
			DERWENT	
61	1	(cholera adj toxin adj B) same subunit\$ same adjuvant same	USPAT:	2002/02/02 40 04
		plasmid	US-PGPUB;	2002/02/26 18:24
			EPO; JPO;	
			DERWENT	
25	8	(enterotoxin same vaccine) and (heat\$labile with II)	USPAT;	2002/00/00 40 05
İ	İ	The state of the s	US-PGPUB;	2002/02/26 18:25
1			EPO; JPO;	
	ļ		DERWENT	
55	62	(cholera adj toxin adj B) same subunit\$ same adjuvant	USPAT:	2002/02/02 40 55
		, and a subunity out to adjuvant	US-PGPUB;	2002/02/26 18:32
			EPO; JPO;	
			DERWENT	
			DEKAAEMI	

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